PROTEOMIC IDENTIFICATION OF S-NITROSYLATED PROTEINS IN *ARABIDOPSIS THALIANA* IN RESPONSE TO PATHOGEN INFECTION

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Nitric oxide (NO) is a highly reactive gas produced by plants under normal growth conditions and under stress situations. The current knowledge on NO-dependent processes illustrates that this molecule can directly influence the activity of target proteins through reversible S-nitrosylation of cysteine thiols. In particular NO plays a crucial role as physiological mediator in plant resistance to pathogens by triggering hypersensitive resistance-associated cell death and by contributing to the local and systemic induction of defence genes

The very transitory nature of this posttranslational modification constitutes an important redox-based regulation mechanism for many proteins, but the technical limitations in characterizing this modification have delayed its study. In order to dissect the NO-signaling pathways during the plant defence responses it is necessary to identify the target proteins and the specific cysteine residues involved. For that purpose we have performed a proteomic identification of S-nitrosylated proteins in *Arabidopsis thaliana* upon infection with the bacteria *Pseudomonas syringae*.

We have used the "biotin switch method" that converts unstable S-nitrosylated cysteines to stably labeled biotinylated cysteines. Afterwards, previously S-nitrosylated proteins can be detected by immunoblot analysis, or further purified by affinity chromatography and identified by means of proteomic methodology using HPLC coupled to a LTQ mass spectrometer.

This approach allowed us to identify an extensive list of proteins from *A. thaliana* cell suspension cultures and leaves in control, GSNO-treated and in *P. syringae*-challenged samples that represent candidates for NO-targets. Among them are proteins involved in defence-and stress-related responses, redox-related proteins, cytoskeleton proteins,

metabolic enzymes and signalling/regulating proteins. In parallel, and in collaboration with other research groups –"the nitrosoteam"– we are developing a methodology to directly identify the modified residues by incorporating an additional digestion step previous to the neutravidin purification. By identifying the NO-targets we hope to get insights into the physiological functions of protein S-nitrosylation during plant defence responses.